REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	APRIL 5 1999	FINAL REPORT JULY 1, 1993 - SEPT. 30, 1998

4. TITLE AND SUBTITLE
DIGESTIVE KINETICS DETERMINES BIOAVAILABILITY OF POLLUTANTS

5. FUNDING NUMBERS
N00014-93-1-1210

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
OFFICE OF NAVAL RESEARCH
800 N. QUINCY ST.
ARLINGTON VA 22217-5000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
DISTRIBUTION UNLIMITED

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The bioavailability of sedimentary contaminants to animals in harbor sediments was addressed by studying the mechanisms by which animals solubilize contaminants during feeding and digestion. Digestive physiology work on many different animal species revealed patterns of enzymes, surfactants and dissolved organic matter that correlate with feeding mode, phyletic position, and diet. Incubation of digestive fluids to dissolve contaminants from polluted sediments was developed to provide numerical estimates of bioavailability, and showed that much higher fractions of total contaminant loading are available than predicted by currently established, aqueous equilibrium approaches. The kinetics of reactions are slow enough that variations in feeding rates will influence overall bioavailability. Experimental manipulations showed mechanisms of bioavailability. Dissolved amino acids, in the form of enzyme proteins and hydrolyzed food, are responsible for solubilization of metals such as copper. At high levels, copper can inactivate digestive enzymes. Metals in sedimentary sulfide minerals were largely impervious to digestive fluid attack. Surfactants are responsible for most solubilization of polycyclic aromatic hydrocarbons (PAH), though other agents also appear to play a role. Bioavailability of both metals and PAH can be limited by saturating the digestive agents responsible for their dissolution.

14. SUBJECT TERMS			15. NUMBER OF PAGES
BIOAVAILABILITY	CONTAMINANTS	PAH	4
SEDIMENTS	METALS		16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

Grant #: N00014-93-1-1210

PRINCIPAL INVESTIGATOR: Dr. Lawrence M. Mayer

INSTITUTION: University of Maine

PROJECT TITLE: Digestive Kinetics Determines Bioavailability of Pollutants

REPORTING PERIOD: 1 July 1993 - 30 September 1998

AWARD PERIOD: 1 July 1993 - 30 September 1998

OBJECTIVES: (1) Determine digestive physiology of deposit feeders; (2) Determine the fraction of total pollutants in sediments released during incubation of polluted sediments with digestive fluid of deposit feeders; (3) Examine partitioning of pollutants in sediments, mechanism of digestive fluid solubilization, and design an *in vitro* method to measure their bioavailability.

APPROACH: (1) Use enzyme and surfactant assays to study digestive capability of a variety of benthic invertebrate animals for lipid component of sediments; identify surfactant compounds in animal guts and digestive products of lipid digestion; (2) Extract gut juices from benthic invertebrates and incubate them with polluted sediments, followed by measurement of pollutants released during the incubation; (3) After determining patterns of digestive agents in various animals, design cocktail of commercially available enzymes and surfactants that can be used to mimic the pollutant release kinetics found in part (2), and to explore phase associations of sedimentary pollutants.

ACCOMPLISHMENTS: We surveyed the digestive enzyme and surfactant activities of 18 benthic invertebrate animals. We focused on deposit feeders from the U.S. east and west coasts, but included carnivores, herbivores, and filter feeders for context. Enzyme and surfactant activities vary across both functional and phyletic categories. Polychaetes have higher enzyme activities than echinoderms, and detritivorous polychaetes have higher protease:lipase ratios than herbivorous and carnivorous species. Intense surfactant activities are found in deposit feeders. Less intense surfactancy is found in carnivores, herbivores and filter feeders. The surfactants from Arenicola marina were found at mM concentrations, consisting of a C9 fatty acid connected via amide bond to an amino acid. The fatty acids show branching and saturation. Feeding experiments with an omnivorous polychaete showed that varying diet induces changes in protease: lipase ratio similar to cross-phyletic patterns. Sediment in the diet had no effect on the surface tension of digestive fluid. Sediment did induce higher surfactant concentrations, as indicated by micelle formation, which can affect contaminant solubilization. Gut fluids have high dissolved organic matter concentrations, with some polychaetes showing dissolved amino acids alone of 5-10%. Amino acids make up roughly half of the dissolved organic matter. Lipids are much less concentrated. Circumstantial and experimental data showed that animals can concentrate dissolved materials (e.g., amino acids, PAH, and radiolabelled polymers) in guts by retaining fluids relative to solids, which can affect subsequent absorption.

Dissolved trace metal concentrations in digestive fluids are very high, with several species having ppm levels. Levels of some metals are proportional to amino acid concentrations, with correlations evident across species, gut section and molecular weight fraction. Metal enrichments in gut fluids, relative to sediments, showed the Irving-Williams order, consistent with soft ligands (e.g., amino acids) as the solubilizing agents.

Metals in contaminated sediments were released during incubation with digestive fluid to a much greater extent than in incubation with seawater. Kinetics of release to digestive fluid were complex among metals and sediments, including even biphasic patterns with opposite signs (early adsorption followed by release). For highly contaminated sediments, the release rate for a metal like Cu is slow enough that incomplete solubilization will occur during gut passage of sediment through the animal. These kinetic patterns will affect the animals' ability to bioaccumulate metals.

The amount of Cu solubilized by different molecular weight fractions of digestive fluid is related to their peptide content. Solubilization is due to complexation by proteins rather than their enzymatic activity. We could mimic Cu release with solutions of off-the-shelf proteins at similar concentrations as are found in digestive fluid. Histidine residues are critical for Cu binding by digestive fluids. Both high molecular weight proteins, representing enzymes secreted by the animal, and lower molecular weight peptides, representing nutritional material solubilized from sediment, were important in releasing Cu from contaminated sediments. Low molecular weight fractions were found to be more effective solubilizing agents, per amino acid residue, likely due to greater exposure to solution. Ion-specific electrodes were used to determine conditional binding constants between Cu and gut ligands, and ranged from 10^{-4} to 10^{-14}, consistent with known histidine affinities for Cu.

Comparison of digestive fluid extraction with the acid-volatile sulfide (AVS) method showed agreement. Digestive solubilization of borderline metals (Cu, Cd, Ni, Zn, Pb) occurred only when these metals were present in concentrations in excess of the AVS, consistent with the relative binding affinities of histidine vs. sulfide.

Solubilization of Cu from sediment can result in inactivation of digestive fluid enzymes. Inactivation occurs at similar ratios of Cu to gut amino acids, across enzyme type and animal species.

Polycyclic aromatic hydrocarbons (PAH) were also found to be solubilized by digestive fluids much more than by seawater. PAH solubilization was found to depend strongly on the solid-solution ratio and sedimentary organic carbon content. At in vivo solid-solution ratios, PAH solubilized by digestive fluid was inversely dependent on sedimentary OC concentration. Increasing the solid-solution ratio decreased the proportion of total PAH extractable by digestive fluid. These two sets of results imply a strong, fugacity-driven partitioning between digestive fluid and sediment. Using dilution experiments that tested for PAH solubility above and below the critical micelle concentration (CMC) of the surfactants in gut fluids, we established an important role of surfactants in PAH solubilization. Solubilities of heavy and light PAH are similar in gut fluid micelles, presumably due to similar limitation by micellar volume. Proteins may also solubilize PAH and other hydrophobic materials (e.g, methyl Hg). PAH solubilization from contaminated sediments is much less than from pure PAH solids, implicating competition for micellar space from other sedimentary lipids. PAH solubilization from highly contaminated sediment is also limited by saturation of the micelles. Hence

bioavailability from such sediments will be more a function of surfactant secretion by the animal than of inherent solubility from the sediment matrix. Repeatedly incubating sediment with fresh batches of gut fluid released similar amounts of PAH, consistent with saturation behavior. The kinetics of PAH solubilization are rapid, reaching completion within a gut residence time. Absorption of PAH by gut walls is dominantly by passive diffusion.

CONCLUSIONS: Digestive fluid contains agents - e.g., amino acids and surfactants - that significantly increase exposure of benthic animals to contaminants during digestion. Variations in bio/chemical properties among animal species and among sediments can explain patterns of bioavailability among animal-sediment combinations. The interactive chemistry of these two "reactants" governs bioavailability.

SIGNIFICANCE: Lack of understanding of bioavailability is the major hindrance in the application of science to contaminant management issues. This study provides a scientific basis for the failure of current regulatory models to explain animal exposure to sedimentary contaminants in harbors. It provides a basis for more accurate understanding and routine determination of contaminant bioavailability and hence risk assessment.

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